THE DETERMINATION OF LARVAL INSTARS OF THE RICE WEEVIL SITOPHILUS ORYZAE L., IN WHEAT

by

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INTRODUCTION AND REVIEW OF LITERATURE

The most destructive pests of stored grain are those insects capable of breaking through the tough seed coat to reach the softer endosperm which serves as food. The rice weevil, Sitophilus (Calandra) oryzae L., is well fitted for such damage because of the slender snout at the tip of which are located powerful mandibles.

Both adults and larvae eat the grain so that it is rendered unfit for human consumption and for seed (Plate I, Figs. 1 & 2). In extreme cases of infestation the grain may become a mass of broken, disintegrated material.

The determination of larval instars of the rice weevil is difficult since they live inside the kernels. Measurement of the head capsule provides a means for exact instar determination.

Three factors are important to determine the time of appearance of a given instar within a culture: period of development, temperature, and moisture of the grain. Howe (1952) has shown that the first appearance of the various larval instars and the emergence of the adult is related to temperature and moisture. At 25°C. and 70 percent relative humidity, the first appearance of each of the four larval instars was 5, 9, 13, and 16 days respectively with adult emergence at 32 days. At 18°C. and 80 percent relative humidity, first appearance of each of the four larval instars was 12, 25, 33, and 46 days respectively with adult emergence in 93 days.

EXPLANATION OF PLATE I

Fig. 1. Dissected wheat kernel showing larval damage; rice weevil larvae is fourth instar.

Fig. 2. Pupal cell of the rice weevil.

PLATE I

Fig. 1

Fig. 2

The experimental work designed for this thesis had a three fold purpose: first, to determine the relative numbers of the various instars present on a given day; second, to obtain head capsule measurements of the four larval instars reared in wheat; and third, to obtain information regarding a possible correlation between instar weight and head capsule width.

Biology

Origin. As early as 196 B. C. mention is made of the ravages of weevils (Cotton, 1920). About the middle of the 18th Century, the rice weevil was found in Europe and was described by Linneaus in 1763 (Cotton, 1920). According to Fletcher (1911), Cotton (1920), and Newman (1927) its origin was believed to be India. Others believe the origin to be some other temperate country (Pruthi and Singh, 1950).

Description of the Adult. The adult rice weevil is characterized by densely pitted circular punctures located on the pronotum and four reddish-orange spots on the elytra.

Description of the Larva. The larva is pearl white, fleshy, and very thick bodied. The ventral outline is approximately straight while the dorsal outline is nearly semicircular. The head is brown with the anterior margins and mandibles much darker. The head is longer than broad and somewhat wedge-shaped, the sides broadly rounded from the middle to the apex, which is slightly angular (Cotton, 1920).

Oviposition. The powerful mandibles, located at the tip of the slender snout, remove bits of the seed coat and endosperm until a cavity has been formed large enough to hold an egg. This cavity is approximately the length of the snout. The cavity is then smoothed on the sides. The female turns about and with the ovipositor thrusts an egg into the cavity. Before the ovipositor is withdrawn, a translucent mass is secreted on top of the egg forming a protective cap or plug for the egg. This translucent cap assumes the color of that portion of the kernel in which it is located (Cotton, 1920).

Development. The egg hatches in approximately three to four days, depending on the temperature and moisture content of the grain. Then it passes through four larval instars characterized by the almost doubling in size in each instar (Plate II, Fig. 1) before entering the pupal stage. The adult develops in about 28 days from the time the egg was first laid in grain of 13.5 percent moisture held at 80°F., and the new adult cuts an emergence hole to the outside (Cotton, 1920). The adults mate within one or two days after emergence according to Cotton (1920); Pruthi and Singh (1950) claim mating occurs as soon as the adults emerge.

Instar Determination. Little information was found in regard to instar determination using head capsule width as the criterion. Cotton (1920) stated that each instar is characterized by the following head capsule widths: 1st Instar--0.22 mm., 2nd Instar--0.32 mm., 3rd Instar--0.48 mm., and 4th Instar--0.64 mm. These measurements were obtained from larvae reared in corn; no measurements were made for larvae reared in wheat.

EXPLANATION OF PLATE II

Fig. 1. The four larval instars of the rice weevil showing the characteristic doubling of size in each instar.

Fig. 2. The rotomatic sifter which was used to separate adults from cultures and to clean the wheat.

PLATE II

Fig. 1

Fig. 2

EQUIPMENT AND MATERIALS

Experimental Insects

Source. The original rice weevil stock came from the Stored Products Insect Section, United States Department of Agriculture, at Manhattan, Kansas.

Maintenance of Stock Cultures. Stock cultures of rice weevils were reared in 75 grams of wheat in wide-mouthed quart mason jars with the lids reversed. Two hundred adults were placed in each jar for three days. This was sufficient time for them to oviposit with a minimum of overlapping instars. At the end of three days the adults were separated from the wheat on a 10-mesh wire screen attached to a removable pan mounted on a rotomatic sifter (Plate II, Fig. 2). The three-day-old culture containing eggs was returned to the original jar and the adults were placed in a fresh jar containing 75 grams of wheat.

These cultures were maintained in a rearing room (Plate III) in which the temperature was held at 80°F. ($\pm$ 2°F.) and the relative humidity at 70 percent ($\pm$ 5 percent). The average period for adult emergence was 28 days.

Age and Sex of Adults. The adults used in this experiment were from four to seven days old. According to Richards (1947), 86 percent of the females contained ripe and fertilized eggs at the four- to five-day level. No attempt was made to sex the adult weevils. According to Cotton (1920), 52 percent of 1000 specimens examined were females and 48 percent, males. Therefore,

EXPLANATION OF PLATE III

Maintenance of stock cultures in the rearing room.

PLATE III

under laboratory conditions approximately 50 percent males and 50 percent females could be expected.

Experimental Wheat

Source. Pawnee wheat from Allen County, Kansas was used.

Moisture Stabilization Equipment. For stabilization of the moisture in the test grain, 100 pounds of wheat were placed in a 55-gallon drum (Plate IV, Fig. 1). The moisture level of the bulk grain was adjusted by addition of distilled water to the desired level of 13.5 percent.

Moisture Determination. Moisture determinations of all grain used in this experiment were made by means of a Type S Steinlite Moisture Tester.

Special Equipment

Aspirator. An aspirator, operated by a Cenco Vacuum pump to which rubber tubing had been attached, was employed in the collection of adults for culture purposes.

Balance. A Roller-Smith balance was used to obtain the weight of 30 larvae of each instar.

Boerner Divider. A Boerner Divider was used to obtain a uniform sample prior to determining the percentage of infestation.

Freezer. A Cold Spot Deep Freeze was used to rid the wheat of insects prior to moisture stabilization.

Microscopic Equipment. Microscopic examination of the infested kernels, their selection, and dissection, and removal and

mounting of the larval head capsule was done with the aid of a low power binocular microscope. Head capsule width was determined using a calibrated ocular micrometer fitted in the eye piece of a monocular compound microscope.

Pinning Forceps. Pinning forceps were used to hold the wheat kernels for dissection.

Rearing Jars. One quart wide-mouthed mason jars with the lids reversed were used to hold the cultures.

Rotomatic Sifter. A rotomatic sifter was used to separate the adults from the wheat and to remove debris from the wheat.

METHODS

Freezing of Bulk Grain

The wheat prior to moisture stabilization was placed in a deep freeze at 0°F. for three days (Plate IV, Fig. 2). According to Robinson (1926) a constant temperature of 0°F. killed all stages of the rice weevil in 1 1/2 hours. Cotton (1950) has shown that a temperature range of from 0°F. to 5°F. killed all stages of many stored grain pests in one day. Allowing for temperature penetration into the center of the 100-pound burlap sack of wheat, and assuming infestation by stored grain insects, other than the rice weevil, might be present, three days was considered to be adequate.

Moisture Stabilization of Bulk Grain

The sack of wheat, after removal from the freezer, was emptied into a 55-gallon drum and the lid tightly secured. The

barrel prior to use had been thoroughly cleaned, fumigated, and aired to remove all traces of the fumigant. Three days were allowed for the wheat to attain room temperature. The wheat was then thoroughly mixed and the moisture content determined using a Steinlite Moisture Meter. In this case the moisture content was below the desired level of 13.5 percent, and water was added according to the following formula: (after Cotton)

$$\frac{\text{100 minus \% of } H_2O \text{ at present level}}{\text{100 minus \% of } H_2O \text{ at desired level}}$$

Drop the first digit of the quotient, which is always one, and take the remainder as the multiple factor. Multiply the weight of the grain in grams by this factor. The answer will be the number of milliliters of distilled water necessary to raise the moisture to the desired level.

The barrel of wheat was set up in this manner in October 1955, and was held at room temperature, approximately 80°F. After the water was added, the barrel was rolled twice daily to keep the grain thoroughly mixed. After seven days the moisture content was again determined and the value and date recorded on the barrel. Since prolonged culturing was not required, the wheat did not have to be retempered.

Cleaning the Grain

Approximately 800-1000 grams of well-mixed 13.5 percent moisture wheat was taken from the tempering barrel and placed in a small enameled basin. Small amounts were then placed on a number

EXPLANATION OF PLATE IV

Fig. 1. Fifty-five-gallon drum (white label) used for moisture stabilization; (other containers are for storage).

Fig. 2. Deep freeze to rid wheat of insect populations.

PLATE IV

Fig. 1

Fig. 2

10 mesh wire screen that was attached to a removable pan mounted on a rotomatic sifter to remove broken kernels and other debris. When cleaned, the wheat was weighed into eight lots of 75 grams each using a triple beam balance and each lot was emptied into a one-quart mason jar. The remainder of the wheat was placed in another container.

Infesting the Grain

Adult weevils were separated from 39-day-old stock cultures by employing a 10 mesh wire screen attached to a removable pan mounted on a rotomatic sifter. After separation the adults were transferred to a procelain enameled pan and 200 adults were counted as they were removed by aspiration. For ease in handling, an electrically operated aspirator was employed in which the suction was obtained by a Cenco Vacuum pump to which rubber tubing had been fitted. In this manner the adults could be collected before they could escape from the pan. The adults were then emptied into a culture jar, containing 75 grams of wheat, and the lid reversed to prevent tight sealing. Two hundred adults were placed in each of eight culture jars which were labeled and placed in the rearing room for three days. At the end of this period, the adults were removed and the wheat returned to the jars. The adults were killed by fumigation. The entire process was repeated every three days for one month.

Determining Extent of Infestation

Eight jars were prepared for each culture and one jar was removed from the rearing room four days after each culture was made. The contents of the jar were run through a Boerner Divider several times to assure a uniformly mixed sample. One hundred kernels were selected at random and placed in a tea strainer. Staining to determine infested kernels was accomplished with acid fuchsin (Frankenfeld, 1948). A tea strainer held the kernels during the staining process. After staining, the kernels were washed in water to remove excess stain and placed on a towel to absorb excess moisture. The kernels were then placed in a petri dish and examined under a binocular microscope for the presence of egg plugs which stained red (Frankenfeld, 1948). Those containing egg plugs were dissected to determine the percentage of infestation.

A four-day-old culture was used to determine the percentage of infestation since at that age only eggs were found to be present and could be easily located. Undoubtedly, a few of the eggs did not hatch but the percentage was thought to be small.

Selecting Cultures of Proper Age

Daily dissections were made at the beginning of this experiment to determine at what age the greatest proportion of the population were in first, second, third, and fourth instars respectively. It was found that 8, 11-12, 15, and 21 days (age from beginning of culture) were required for instars one through four

respectively. Eleven-day-old cultures were used in this experiment for obtaining of second instar. All instar determinations were based on head capsule width.

Dissection of Infested Kernels

Cultures of 8, 11, 15, and 21 days were removed from the rearing room, stained and infested kernels selected as previously described. Infested kernels of 8 and 11 days old were held by pinning forceps applied to the end opposite the egg plug which in all cases faced upward. With a scapel, a cut was made parallel to the egg plug base and a portion of the kernel, containing the egg plug, was removed. If frass was not found, the egg plug was dissected to see if the egg had hatched. If frass was found, further dissection was done by removing small portions of the kernel with the aid of a number one dissecting pin until the larva was exposed. Removal was accomplished by enlarging the cavity occupied by the larva and inverting the kernel over a petri-dish, at a height of approximately one-eighth inch or less, and the larva dropped into the dish. In this manner there was no danger of puncturing the larval skin or damaging the head capsule. Larvae from 15- and 21-day-old cultures were removed in the same manner but dissection of the kernel differed slightly. The kernel was gripped with the pinning forceps so that the ventral crease faced upward. With a scapel, a cut was made along this crease and the two halves separated with forceps. Larvae of this age had eaten a sufficiently large amount of the endosperm so that their position in the kernel was easily found.

Calibration of Ocular Micrometer

Installation. The ocular was removed from a Spencer compound monocular microscope and the top unscrewed. An ocular micrometer was fitted in the center portion so that it rested on top of the hollow cylindrical lock nut holding the base lens in place. The top was replaced and tightly secured and the ocular placed back into the microscope tube.

Calibration. When viewed, through the microscope, the micrometer was found to consist of horizontal and vertical lines crossing each other and forming 100 squares, 10 deep and 10 wide. A calibrated slide was employed to obtain the number of millimeters covered by ten squares as follows: the base line of the slide was alligned with the left vertical line on the micrometer and by reading from left to right the distance covered was found to be 0.7 mm. Therefore, one square equalled 0.07 mm. To check the latter, a calibrated slide divided into 0.01 mm. was employed and the value for one square was found to be exactly 0.07 mm.

Head Capsule Preparation and Measurement

Removal and Mounting of Head Capsule. After approximately 30 larvae of desired age were obtained from the infested wheat, they were prepared for head capsule measurement. Singly, each larva was placed on a standard microscope slide and examined under the low power objective of a binocular microscope. The larval head was removed using a number one insect pin as the dissecting instrument. The severed head was then transferred to

another microscope slide, to which a thin film of Canada balsam had been applied, and arranged in such a manner that the vertex and margins were completely visible when viewed from above under low magnification. After approximately 30 head capsules had been mounted, they were observed under medium magnification and once again alligned so that complete visibility was assured.

Measurement. The slide containing the mounted head capsules was transferred to a compound monocular microscope containing an ocular micrometer which had been calibrated as previously described. Each head capsule was then measured in the following manner: the third horizontal line of the micrometer was alligned with the base of the mandibles so that they were parallel. Once accomplished, the left margin of the head capsule was alligned with the left vertical line of the micrometer so that the most extreme edge of the capsule was just covered by this line. The number of squares, covered by the greatest width of the head capsule, were then counted and where the right margin of the capsule fell between two squares that distance was approximated to the nearest tenth square. The number of squares, to the nearest tenth, covered by each capsule was then multiplied by the calibration constant, 0.07 mm. The reading obtained was the head capsule width in millimeters. At least 100 head capsules were measured in this manner for each of the four culture ages previously described.

Weighing Larvae and Determining Head Capsule Width

Removing and Handling Larvae. The larvae were removed from cultures of 8, 11, 15, and 21 days, and handled as previously described, but with the following exception. A total of 30 larvae in lots of five were removed from the infested wheat and placed in a covered petri-dish. This prevented excessive loss in weight which could occur due to moisture loss from the body.

Weighing Larvae. Each of the five larvae were brushed gently with a fine camel's hair brush to remove any material clinging to the body surface. Each larva was then transferred to the weighing arm of a Roller-Smith balance of five milligram capacity, and the weight determined and recorded.(Plate V).

Handling Weighed Larvae. Each larva, immediately after weighing, was placed in a small gelatin capsule that had previously been numbered. A total of thirty capsules were so numbered.

Removal, Mounting, and Measuring Head Capsules. The larva from each numbered capsule was treated singly on a glass microscope slide and the head removed, mounted, and measured as previously described. By individual handling, confusion was eliminated and the head capsule width was determined for a larva of known weight.

EXPLANATION OF PLATE V

The author manipulating the Roller-Smith balance used for obtaining larval weights.

PLATE V

RESULTS AND DISCUSSION

Under experimental conditions previously described, this investigation was designed to show the following: (1) number of days from the starting of the culture to obtain the greatest number of larvae in instars one through four respectively; (2) larval head capsule widths for each instar; and (3) correlation between larval weight and head capsule width.

Age of Culture

By daily dissections of 10 kernels it was found that the majority of the population examined were in instars one through four at 8, 11, 15, and 21 days (± one day) respectively from the beginning of the culture. Table 1 shows the percentage of the population found in each instar at the above days. The time required for the first appearance of each of the four instars closely followed those reported by Cotton (1920) and those reported by Howe (1952) at 25°C. and 70 percent relative humidity.

Table 1. The occurrence of rice weevil larval instars one through four in 8-, 11-, 15-, and 21-day-old (± one day) cultures in 13.5 percent (± 0.3 percent) moisture wheat at 80°F. (± 2°F.).

Age of culture (days)	Total larvae measured	Instar ------- percentage				
		1st.	2nd.	3rd.	4th.	Uncertain
8	128	98.4	1.6	---	---	---
11	121	21.5	72.8	3.2	---	2.5
15	146	---	5.5	82.6	10.5	1.4
21	116	---	---	---	100.0	---

Width of Head Capsule

A greater overlap was observed in the second and third instars than in the first and fourth instars (Plate VI). The range of head capsule widths for each instar together with the average width and uncertain widths is shown in Table 2. Uncertain widths refers to a few measurements that could not be assigned to proper instars. When Dyars' rule (Imms, 1924) was applied to the average head capsule measurements, it was found that the calculated and weighted average values were identical while the observed mode differed by 0.01 mm. for third and fourth instars but was identical to the calculated values for the first and second instars (Table 3).

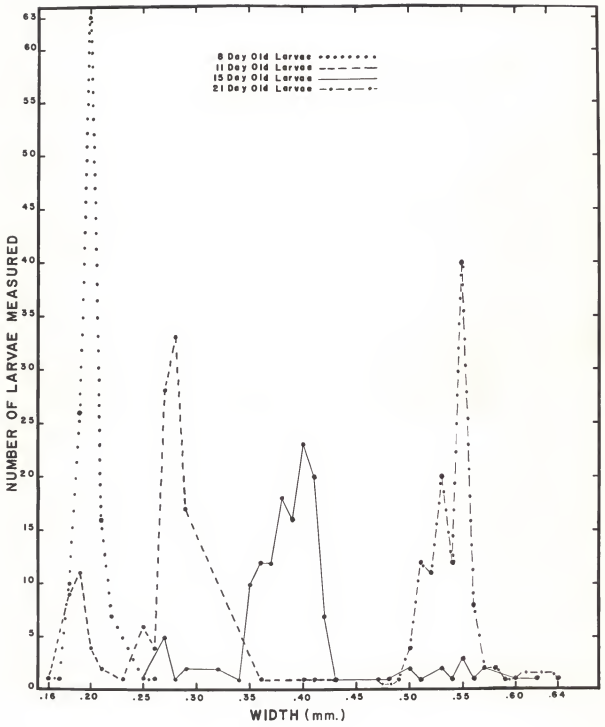
The overlap indicated in Plate VI may be due to the three-day oviposition period permitted the adults before they were removed from the cultures.

Cotton (1920) reported head capsule measurements of 0.22., 0.32 mm., 0.48 mm., and 0.64 mm. for the four larval instars. The weighted average (Table 2) obtained in this study for each instar was lower in some cases than those of Cotton's. Possible explanations include the following: first, the larvae measured by Cotton were obtained from corn whereas larvae in this experiment were obtained from wheat; second, Cotton (1920) may have used the large strain of rice weevil which breeds and lives in corn whereas the small strain lives and breeds in wheat (Richards, 1944; Birch, 1954).

EXPLANATION OF PLATE VI

A comparison of larval head capsule widths from 8-, 11-, 15-, and 21-day-old cultures maintained in 13.5 percent (± 0.3 percent) moisture content wheat held at 80°F. (± 2°F.).

PLATE VI


8 Day Old Larvae
11 Day Old Larvae
15 Day Old Larvae
21 Day Old Larvae
NUMBER OF LARVAE MEASURED
0
5
10
15
20
25
30
35
40
45
50
55
60
63
.16
.20
.25
.30
.35
.40
.45
.50
.55
.60
.64
WIDTH (mm.)

An error in measurements of the head capsules was unlikely as duplicate measurements were made, using another calibrated ocular micrometer. The average values differed by 0.01 mm.

Table 2. Head capsule widths of rice weevil larvae of the first through the fourth instar reared on wheat.

Instar	Number of larvae measured	Head capsule width: Weighted average (mm.)	Range (mm.)	Uncertain range (mm.)
1	128	0.20	0.16-0.22	0.23-0.24
2	121	0.28	0.25-0.29	0.30-0.33
3	146	0.39	0.34-0.43	0.44-0.48
4	116	0.54	0.49-0.64	---------

Table 3. A comparison between the calculated head capsule widths for the four instars of rice weevil larvae using Dyars' rule.

Instar	Dyars' rule: Calculated width (mm.)	Observed widths: Mode (mm.)	Weighted average (mm.)
1	----------	0.20	0.20
2	(1.39 x 0.20) = 0.278	0.28	0.28
3	(1.39 x 0.28) = 0.389	0.40	0.39
4	(1.39 x 0.39) = 0.542	0.55	0.54

Relation of Larval Weight to Head Capsule Width

The results of this phase of the study showed a positive relationship between larval weight and head capsule width (Plate VII). For first and second instar larvae there is associated with each increase in weight of 1 milligram on the average, an increase of 1.253 millimeters in head capsule width significant to the 1 percent level. For larvae of the third and early fourth instar there is associated with each milligram of weight increase, on the average, an increase of 0.2492 mm. in head capsule width significant to the 1 percent level. For late fourth instar larvae there is an increase of 0.0384 mm. in head capsule width, on the average, for each milligram of weight increase significant to the 5 percent level.

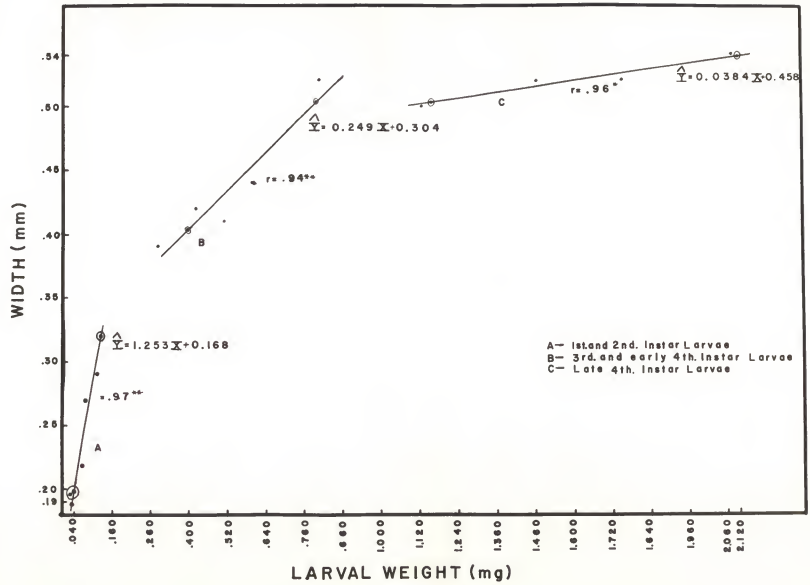
Bodenheimer (1932) stated that in holometabolous insects the weight increase per instar was extremely variable. This was shown to be true in late third and early fourth instars, but not to the extent of three to 17 times the initial weight as found by Bodenheimer.

The weight could possibly be used as an indication of instar for first, second, and late fourth instar larvae. Third and early fourth instar larvae could not be identified by this method since their weights were found to overlap. The following reasons for this overlap are suggested. Since the adults were allowed to oviposit for three days before removal, therefore, at the culture periods of 8, 11, 15, and 21 days, there could be a one-day variation. Another possibility is in the amount of nutrients available

EXPLANATION OF PLATE VII

A comparison between larval instars of the rice weevil to show the correlation between larval weight and head capsule width. (Differentiation of early and late fourth instar larvae is based on weight.)

PLATE VII


WIDTH (mm)
.54
.50
.45
.40
.35
.30
.25
.20
.19
Ŷ= 1.253X+0.168
r=.97**
A
Ŷ= 0.249X+0.304
r=.94**
B
C
r=.96**
Ŷ= 0.0384X+0.458
A— 1st and 2nd. Instar Larvae
B— 3rd. and early 4th. Instar Larvae
C— Late 4th. Instar Larvae
.040
.100
.160
.220
.280
.340
.400
.460
.520
.580
.640
.700
.760
.820
.880
1.000
1.120
1.240
1.360
1.480
1.600
1.720
1.840
1.960
2.080
2.120
LARVAL WEIGHT (mg)

to the larvae. Also a freshly moulted larva might weigh less than a larva prior to moulting. It was observed that prior to moulting and immediately after moulting all feeding and larval movement ceased.

SUMMARY AND CONCLUSIONS

This investigation was designed with a three-fold purpose: (1) to obtain information on the percentage of individuals of the various instars present on a given day; (2) to obtain head capsule measurements of the four larval instars reared in wheat; and (3) to obtain information regarding a possible correlation between instar weight and head capsule width.

Stock cultures of rice weevils were reared in wide-mouthed one quart mason jars containing 75 grams of Pawnee wheat held at 13.5 percent (± 0.3 percent) moisture content and at 80°F. (± 2°F.). Two hundred adult weevils, approximately four to seven days old, obtained from stock cultures, were placed on the wheat for three days after which time they were removed by screening. Kernel examination, larval removal, head capsule measurement, and weight determinations were made at the end of 8, 11, 15, and 21 days from date of culture.

It was found that an eight-day culture contained 98.4 percent of the population in first instar with 1.6 percent in second instar. Eleven-day cultures contained 72.8 percent second instar larvae with 21.5 percent in first instar and 3.2 percent in third instar. At this age 2.5 percent were found that could not be definitely distinguished as to instar because so few individuals

occurred within these limits. Fifteen-day cultures showed 82.6 percent of the population to be in third instar with 5.5 percent in second instar and 10.5 percent in fourth instar. At this age, 1.4 percent were termed uncertain as to instar. Twenty-one-day cultures showed 100.0 percent of the population to be in fourth instar.

The range of head capsule widths for each instar was determined to be as follows:

First instar--between 0.16 and 0.22 mm., with 0.23 to 0.24 mm. termed as uncertain.

Second instar--between 0.25 and 0.29 mm., with 0.30 to 0.33 mm. termed uncertain.

Third instar--between 0.34 and 0.43 mm., with 0.44 to 0.48 mm. termed uncertain.

Fourth instar--between 0.49 and 0.64 mm., with no uncertain range.

The weighted average for each instar was found to be:

First instar----0.20 mm.

Second instar----0.28 mm.

Third instar----0.39 mm.

Fourth instar----0.54 mm.

When Dyars' rule was applied to the average head capsule measurements, it was found that the calculated and weighted average values were identical while the observed mode differed by 0.01 mm. for third and fourth instars but was identical to the calculated values for the first and second instars.

When a correlation was made between larval weight and head capsule width for first and second instars, third and early fourth instars, and late fourth instar the following results were obtained. In first and second instars, for each increase of 1.0 mg. in weight there is associated with this increase, on the average, an increase of 1.253 mm. in head capsule width. For each increase of 1.0 mg. in weight for third and early fourth instars there is associated, on the average, an increase of 0.2492 mm. increase in head capsule width. For late fourth instar larvae there is associated with each 1.0 mg. in weight increase, on the average, an increase of 0.0384 mm. in head capsule width.

Larval weight may possibly be used as a criterion for instar determinations in first, second, and late fourth instar. Third and early fourth instars have too large a weight overlap to enable weight to be used for certain instar identification.

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THE DETERMINATION OF LARVAL INSTARS OF THE RICE WEEVIL SITOPHILUS ORYZAE L., IN WHEAT

by

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The most destructive pests of stored grain are those insects such as the rice weevil, which are capable of breaking through the tough seed coat to reach the softer endosperm which serves as food.

Three factors are important in obtaining a given instar from a culture: period of development, temperature, and moisture of the grain.

The experimental work designed for this thesis had a threefold purpose: (1) to obtain information on the greatest number of individuals of a specific instar present on a given day; (2) to obtain head capsule measurements of the four larval instars reared in wheat; and (3) to obtain information regarding a possible correlation between instar weight and head capsule width.

Stock cultures of rice weevils were reared in wide-mouthed one quart mason jars with the lids reversed. Each jar contained 75 grams of 13.5 percent ($\pm$ 0.3 percent) moisture content wheat. Two hundred adults were placed in each jar where they remained three days. At the end of three days the adults were separated by screening and the wheat returned to the original jar. The adults were placed in a fresh jar containing 75 grams of wheat. All cultures were maintained in a rearing room with the temperature held at 80°F. ($\pm$ 2°F.).

The grain was rendered free of insects by exposure for three days at 0°F. in a deep freeze after which the moisture content was brought up to 13.5 percent.

Two hundred adults of four-to seven-days-old were placed in each of eight culture jars containing 75 grams of 13.5 percent

(≠ 0.3 percent) moisture wheat. All cultures were placed in a rearing room for three days after which the adults were removed and the wheat returned to the original jars.

Four days from date of culture one of the eight culture jars was removed from the rearing room and the entire contents run through a Boerner divider several times to obtain a uniformly mixed sample. One hundred kernels were selected at random and stained with acid fuchsin. Kernels containing egg plugs were dissected to determine the percentage of infestation.

Daily dissections of 10 kernels were made at the beginning of this experiment to determine at what age the greatest proportion of the population were in instars one through four respectively.

After approximately 30 larvae had been removed from infested wheat of desired age, they were prepared as follows: singly, each larval head was removed and transferred to a microscope slide containing a thin film of Canada balsum. When all heads were so mounted the slide was transferred to a microscope containing a calibrated ocular micrometer. The greatest width of the head capsule was measured to the nearest tenth square and the value multiplied by the calibration constant (0.07 mm./square) and the reading obtained was the head capsule width in millimeters.

Five larvae were transferred singly to the weighing arm of a Roller-Smith balance and weight determined. The larval head was removed and measured as previously described. The entire process was repeated until 30 larvae had been measured.

It was found that eight-day cultures contained 98.4 percent of the population in first instar with 1.6 percent in second instar. Eleven-day cultures contained 72.8 percent second instar larvae with 21.5 percent in first instar and 3.2 percent third instar. At this age 2.5 percent were termed uncertain because they could not be definitely distinguished as to instar because so few individuals occurred within these limits. Fifteen-day cultures showed 82.6 percent of the population to be in third instar with 5.5 percent in second instar and 10.5 percent in fourth instar. At this age, 1.4 percent were termed uncertain as to instar. Twenty-one-day cultures contained 100.0 percent of the population in fourth instar.

The range of head capsule widths determined for each instar was as follows: first instar 0.16-0.22 mm.; second instar 0.25-0.29 mm.; third instar 0.34-0.43 mm.; and fourth instar 0.49-0.64 mm. Measurements between these ranges were termed uncertain. The weighted average head capsule widths for instars one through four respectively was: first instar 0.20 mm.; second instar 0.28 mm.; third instar 0.39 mm.; and fourth instar 0.54 mm.

When Dyars' rule was applied to the average head capsule measurements, it was found that the calculated and weighted average values were identical while the observed mode differed by 0.01 mm. for the third and fourth instars but were identical to the calculated values for the first and second instars.

It was found that for larvae of the first and second instars, for each additional increase of 1.0 mg. in weight there is

associated with this increase, on the average, an increase of 1.253 mm. in head capsule width. For each increase of 1.0 mg. in weight for third and early fourth instars there is associated, on the average, an increase of 0.2492 mm. in head capsule width. For late fourth instar larvae there is associated with each 1.0 mg. in weight increase, on the average, an increase of 0.0384 mm. in head capsule width.

Larval weight can be used as a criterion for instar determinations in first, second, and late fourth instar larvae. Third and early fourth instar larvae show too great a weight overlap.